Effect of Cholestyramine on the Fecal Excretion of Intravenously Administered Cholesterol-4-^{14}C and its Degradation Products in a Hypercholesterolemic Patient

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ABSTRACT The effect of cholestyramine on the fecal excretion of bile acids and neutral sterols was measured in a hypercholesterolemic patient on a low fat, high polyunsaturated fatty acid–containing diet after the intravenous injection of cholesterol-4-^{14}C. A significant (16%) lowering of serum cholesterol concentration was accompanied by a 3.2-fold increase in fecal bile acid excretion but no change in neutral sterol output. The increased bile acid loss was adequate to account for the observed fall in serum cholesterol level. The implications of these findings were discussed.

INTRODUCTION

Cholestyramine is a water insoluble, solid, quaternary ammonium, anion-exchange resin with a polystyrene skeleton and a molecular weight of over 1,000,000 which binds bile salts both in vitro and in vivo (1). It is used as the chloride salt, which is neutral in reaction, and is not absorbed or digested in the gastrointestinal tract (2). When given in the appropriate dosage it has been shown to reduce the blood cholesterol levels in hypercholesterolemic cockerels (2, 3), dogs (4), rabbits (5), and man (6–9), and in normocholesterolemic cockerels, dogs (1), and man (9). It has been reported to produce increased fecal excretion of both bile acids and neutral sterols in the dog (1) and the pig (10), and of bile acids alone in the rat (11, 12). Carey and Williams (13) found a marked increase in fecal deoxycholic acid excretion in a normal man during the first 4 days of cholestyramine ingestion (at a dose of 10 g/day), but their study was not designed to measure total bile acid excretion, which is considerably greater than the amount of the one bile acid studied in their subject (14). Hashim and Van Itallie (9) reported a marked increase in fecal bile acid excretion with no significant change in neutral sterol output in two female hypercholesterolemic patients and in one normal man given 13.3 g of the resin daily. Although the recovery of bile acids during the control period (150–289 mg/day) was considerably larger than that reported by Carey and Williams (31.2 mg/day), it fell far short of the values obtained by isotopic techniques (15–18). Since the intestinal microflora may alter the structure of the bile acids and sterols there may be a loss of some of the fecal end products of cholesterol catabolism in the extraction and separation processes commonly used (19–21). However, by labeling the body cholesterol "pool" with cholesterol-4-^{14}C such losses can be minimized because the radioactivity may be detected even though the individual compounds have not been isolated and identified. Therefore, it seemed worthwhile to study the effect of the resin on the fecal excretion of cholesterol catabolites by using isotopic methods. This paper describes the effect of cholestyramine on the fecal excretion of intravenously administered cholesterol-4-^{14}C and its degradation products in a male hypercholesterolemic patient under

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carefully controlled conditions in a metabolic study unit.

METHODS

Experimental design. A 45 yr old, 61.8 kg male hypercholesterolemic patient with tendon xanthomas and xanthelasma, who had been followed in our out-patient clinics for the preceding 10 yr, was admitted to the metabolic study unit in the University of Minnesota Hospital. He was fed a 2200 calorie diet (374 g of carbohydrate, 77 g of protein, 42 g of fat) which provided 17% of the total calories as fat and which contained 120 mg of cholesterol and 220 mg of plant sterols daily. The carbohydrate was provided by breads, cereals, sugar, fruits, and vegetables. The protein was provided mainly by fish, lean poultry, and skimmed milk. Of the dietary fat content (42 g/day) 11 g was contained in poultry and fish and 31 g was given as safflower oil, either added directly to such foods as salads and casseroles or incorporated (with buttermilk and skimmed milk powder) into a spread used in lieu of butter. Of the total aggregate fatty acids in the diet there were 7.3 g of saturated and 26.4 g of polyunsaturated fatty acid glycerides per day, giving a polyunsaturated-to-saturated (P:S) ratio of 3.6 to 1. Dietary control was insured by prohibiting the ingestion of any food except that provided in the diet and was confirmed by keeping daily records of the patient's weight and the food he consumed.

The study was divided into three parts: an initial *control period* of 23 days during which clinical studies were performed and diet therapy was instituted; experimental *period A* of 20 days duration during which fecal excretion of bile acids and neutral sterols was measured while on diet therapy alone; and *period B* of 20 days duration during which fecal steroid excretion was measured while cholestyramine (in doses of 3 g four times daily) was added to the diet. On the 5th day of the control period the patient was given a single intravenous injection of 35 μc of cholesterol-4-^{14}C as a suspension in saline. This injection was prepared by dissolving the appropriate amount of labeled crystalline cholesterol of high specific activity (30 mc/mmole, with no added "cold-carrier" cholesterol) in 2 ml of absolute ethanol and suspending this solution in 30 ml of saline solution by vigorous shaking immediately before injection into the patient. All stools were collected after the 5th day and kept frozen in individual plastic bags. The collections during the control period were made to familiarize the patient with this procedure and to evaluate his bowel habit. 4-day pooled feces collections were used for neutral sterol and bile acid analysis in periods A and B. During the 10 yr preceding this study blood samples had been obtained at intervals for the determination of serum cholesterol concentration. Throughout the experiment fasting blood samples were obtained twice weekly. The patient was ambulatory and symptom-free during the course of the study.

Methods of analysis. Total serum cholesterol concentration was determined on the fasting blood samples by the method of Abell, Levy, Brodie, and Kendall (22). Aliquots of the petroleum ether extract obtained by this method (containing "total" cholesterol) were placed in glass counting bottles, the solvent was evaporated, the sterols were dissolved in 0.3% diphenyloxazole in toluene, and the ^{14}C radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer, Packard Instrument Co., Downers Grove, Ill. After the radioactivity was counted, duplicate aliquots from each counting bottle were removed, the solvent was evaporated, and the amount of cholesterol present was determined spectrophotometrically with the Liebermann-Burchard color reaction. The specific activity of the serum total cholesterol was determined by dividing the radioactivity (in disintegrations per minute) by the amount of cholesterol (in milligrams) in each counting bottle. All serum samples were subjected to duplicate extraction and radioactivity measurements.

The stools passed during each 4 day period were pooled and homogenized in an electric blender with measured amounts of distilled water to obtain a slightly flowing consistency. The lipids were extracted by a modification of the method we had previously reported (16). Weighed aliquots (8–12 g) of the homogenized sample were extracted with boiling alkaline ethanol solution for 2 hr, with a vertical reflux condensor to prevent loss of solvent. The extract was separated from the nonextractable residue by vacuum filtration through a sintered-glass filter, and the residue was washed three times with aliquots of fresh hot 95% ethanol to prevent loss of extracted lipids. The volume of the extract was reduced to 20 ml, and this amount was extracted four times with 40-ml aliquots of petroleum ether (60–70°C boiling point) in a separatory funnel. The four petroleum ether layers were then combined, and the volume was reduced to 40 ml and extracted twice with 10-ml aliquots of 0.1 N aqueous NaOH. The aqueous layers were added to the ethanol fraction, placed in a 100 ml volumetric flask, and made up to volume with ethanol. The remaining petroleum ether layer was placed in a 50 ml volumetric flask and made up to volume with petroleum ether. The three fractions of the stool sample thus obtained were the residue, the aqueous ethanol layer containing the bile acids (as salts), and the petroleum ether layer containing the unsaponifiable material (neutral sterols).

Duplicate aliquots of these fractions and the homogenized stool samples were used to measure ^{14}C radioactivity. The carbon was converted to CO_2 by the wet-carbon combustion method of Van Slyke, Folch, Plazin, and Weisiger (23, 24), collected in a reservoir, and absorbed into phenethylamine by a method devised by the authors (25); the ^{14}C was measured in a liquid scintillation counter with a solution of 0.3% diphenyloxazole in toluene containing the phenethylamine and methanol. (A solution containing 2 ml of 1:1 phenethylamine: methanol and 10 ml of 0.3% diphenyloxazole was adequate for up to 6 mmoles of carbon dioxide.)

Calculation of fecal excretion rates. The method used to calculate the amount of cholesterol excreted in the feces has been previously reported (16) and was similar to that used by Hellman, Rosenfeld, Insull, and Ahrens (26), by Hellman and Rosenfeld (27), and by Frantz,

Carey, Moss, Eckert, Goldfarb, and Katz (15). The method is based on the fact that the fecal end products of cholesterol metabolism have specific activities that closely parallel those of the serum cholesterol (15, 17, 27). Since the specific activities of plasma free and ester cholesterol are very similar after the first 2–5 days and decline at essentially similar rates (26, 28–31), the use of the serum total cholesterol specific activities instead of the free cholesterol values will give equally satisfactory results provided the first 5 days are excluded from these calculations. The patient was given a gelatin capsule containing small colored glass beads; the length of time required for a complete transit of the gastrointestinal tract was found to be approximately 24 hr. The values of the serum cholesterol specific activity were plotted against time on a semilogarithmic scale, and the value at 24 hr before the midpoint of each 4 day stool collection period was obtained. By dividing this value (in disintegrations per minute per milligram) into the total radioactivity in the particular stool sample or its extracted fractions (in disintegrations per minute) the amount of neutral sterols or bile acids in the sample was obtained (in milligrams). Since 4-day pooled collections were used, the average daily fecal excretion rates were determined by dividing these values by 4.

RESULTS

Serum cholesterol level. During the 10 yr before this study the patient's total serum cholesterol levels had ranged from 400 to 500 mg/100 ml and the average value during the month before this study was 439 mg/100 ml (range from 407 to 488 mg/100 ml). During the part of the study when the low fat high P : S ratio diet was given (control period and period A) there was a slight decrease in serum cholesterol level to an average value of 419 mg/100 ml (range from 373 to 458 mg/100 ml) the difference between the average value during the diet period and the 1 month period before the study was not statistically significant ($P > 0.25$). The mean value and the range of values for the serum cholesterol concentration before, during, and after the study are shown in Fig. 1, together with the individual values during the two fecal steroid measurement periods A and B. When cholestyramine was added to the diet therapy (in period B) there was a 16 % reduction in the

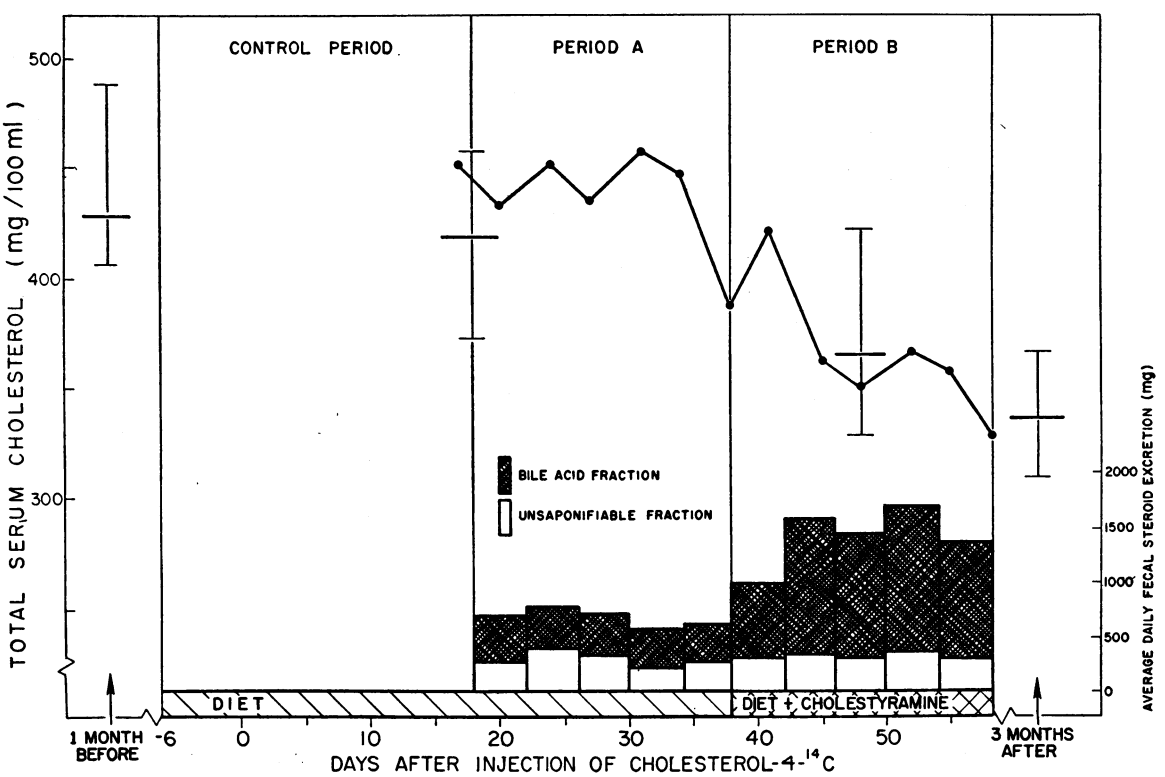

FIGURE 1 Serum total cholesterol concentration and average daily fecal sterol and bile acid excretion in patient J. I. The horizontal bars indicate the mean serum cholesterol concentration for the period shown and are bracketed by the observed range of values. (See Results section.)

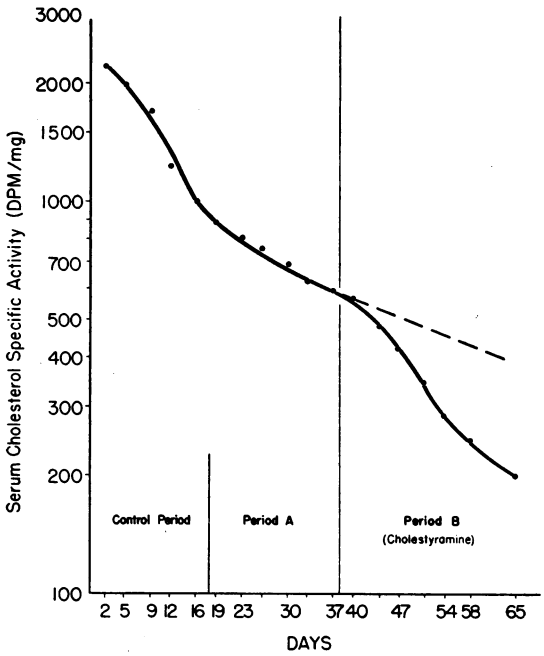

FIGURE 2 Semi-log plot of serum total cholesterol-^{14}C specific activity values in patient J. I.

cholesterol level to an average of 365 mg/100 ml (range 329–422 mg/100 ml), and it appeared to be continuing to decline at the end of the study period. The difference between the means of period A (diet alone) and period B (diet plus cholestyramine) was statistically highly significant ($0.005 > P > 0.001$). If the last 16 days of period B is compared with the diet-only period (A), the difference is even greater (354 vs. 419 mg/100 ml, and $P < 0.001$). The patient continued on the diet and cholestyramine therapy after his discharge from the hospital, and there was a further fall in serum cholesterol during the 3 months after the study to an average of 336 mg/100 ml (range 310–367 mg/100 ml). Therefore, the effect of cholestyramine in lowering blood cholesterol level continued in this patient when he returned to his normal environment and probably liberalized his diet somewhat from the rigid control of the metabolic study unit.

Serum cholesterol specific activity. Fig. 2 shows the serum total cholesterol specific activity values plotted against time on a semilogarithmic graph. It can be seen that after an initially rapid fall in values the line becomes essentially a straight one, indicating a single exponential rate of decline after about the 26th day. The rate of decline in the specific activity values is a function of the net loss of labeled cholesterol and the net gain of unlabeled cholesterol by the compartment being sampled (the blood-liver "pool"). In a dynamic steady state of equilibrium the slope of the specific activity–time decay curve reflects in part the cholesterol turnover rate, although the size of the blood-liver cholesterol "pool" and the rate of exchange with other tissue cholesterol "pools" are also involved in the determination of this slope. During cholestyramine therapy an abrupt change was noted in this curve with a more rapid rate of decline in specific activity values than in the preresin period. In the latter part of the resin period (period B) the points again tended to form a straight line having a greater negative slope (half time = 30 days) than in the preresin period (half time = 72.5 days), suggesting that a new dynamic steady state had been established at a greater level of cholesterol turnover. The measurements of fecal bile acid and neutral sterol excretion confirm this conclusion.

Fecal bile acid and neutral sterol excretion. The values for the fecal excretion of cholesterol and its degradation products in the 10 pooled 4-day collections in experimental periods A and B (samples 1–10) are listed in Table I. This table gives the average daily fecal excretion of cholesterol from the freely miscible cholesterol pool (in milligrams) in the two major fractions of the stool extracts (bile acid and unsaponifiable fractions), the total excreted (sum of these two fractions), and the total amount obtained by combustion of the homogenized stool sample itself (total homogenate). Statistical analysis of the data for periods A and B separately demonstrated that there was no significant difference ($P > 0.40$) between the total when measured directly (total homogenate) and the total when the sample was extracted and separated into bile acid and neutral sterol fractions (extraction method). Statistical evaluation of the amounts excreted in the two fractions demonstrated that there was a significantly greater total steroid excretion during cholestyramine therapy than during the diet alone ($P < 0.001$), and that this was due entirely to the increase in bile acid excretion during cholestyramine therapy ($P <$

TABLE I

*Average Daily Fecal Excretion of Cholesterol-4-^{14}C and its Degradation Products**

Sample	Unsaponifiable fraction (U)	Bile acid fraction (B)	Total (U + B)	Total (homogenate)
Period A				
1	292	349	645	599
2	388	353	788	740
3	299	351	685	795
4	214	287	520	608
5	220	308	562	676
Mean ± 1 SD	283 ± 73	330 ± 30	640 ± 105	684 ± 85
Period B				
6	285	655	949	1078
7	323	1225	1584	1619
8	293	1106	1437	1663
9	376	1305	1732	1888
10	322	1089	1429	1718
Mean ± 1 SD	320 ± 36	1076 ± 253	1426 ± 294	1593 ± 306
Value of P (difference between A & B)	$0.20 > P > 0.10$	0.001	0.001	0.001

* In patient J. I., expressed in mg/day.

0.001) with no significant change in the unsaponifiable fraction ($0.20 > P > 0.10$).

DISCUSSION

The isotope balance method used in this study is based upon the principle that the 4-carbon atom of the administered cholesterol-4-^{14}C is not altered by body metabolism or passage through the intestinal tract (26, 28, 32, 33), and therefore any radioactivity recovered in the feces must ultimately have been derived from the plasma cholesterol. Labeled cholesterol given intravenously as a suspension is rapidly removed from the plasma, released back into the circulation in the form of lipoproteins during the following several hours, and is essentially indistinguishable from labeled cholesterol given as lipoproteins (prepared either in vitro or in vivo) after the 3rd day (34). The specific activities (SA) of plasma free and ester cholesterol have been shown to be very similar after the first 2–5 days (26, 28, 31), and we have shown (unpublished study) that the serum total cholesterol specific activity is essentially the same as the serum free SA after the 5th day. The specific activities of the fecal end products of cholesterol metabolism have been shown to approximate the serum cholesterol specific activity at the time these products are secreted into the lumen of the intestine (15, 17, 27, 35). Although we have not measured fecal bile acid or sterol specific activities in this patient, we have no reason to believe that the fecal end product specific activities fail to parallel that of the plasma cholesterol during the time that fecal steroid excretion is augmented (cholestyramine period). In fact Wood, Shioda, and Kinsell (35) have found that the specific activities of biliary free cholesterol, cholic acid, and chenodeoxycholic acid continue to approximate that of the plasma free cholesterol during a period of increased cholesterol turnover (with a greater slope in the plasma cholesterol specific activity curve) resulting from a change in the type of ingested fat. Since the isotope balance method gives values for the endogenous sterol excretion, dietary sterols should not interfere in the measurements. Recently Ahrens (36) has reported finding significant losses of fecal neutral steroids in some (but not all) patients when the isotope balance method was used, which he attributed to degradation of the sterols to nonsteroidal compounds within the small intestinal lumen. Because of this finding we cannot rule out the possibility that the isotope balance method used in the present study

may underestimate the amount of fecal neutral sterol excretion.

Since bile acids are the oxidative end products of cholesterol catabolism in the liver (14, 32) they exert an important influence on body cholesterol metabolism. Hepatic bile acid synthesis is regulated in part by the concentration of bile acids in the portal vein blood reaching the liver, and any substance or procedure which interferes with the normal reabsorption of bile acids from the intestine will elicit a compensatory increase in hepatic bile acid synthesis (14). Since the cholesterol pool of the blood has a rapid rate of exchange with that of the liver (28, 37), the rate of conversion of cholesterol to bile acids in the liver may influence the blood cholesterol concentration. If the augmented bile acid loss from the body (with resulting increased catabolism of cholesterol) exceeds the body's ability to increase de novo cholesterol synthesis, a reduction in the size of the blood-liver cholesterol pool may result. In both the rat (11, 12) and the pig (10) the ingestion of cholestyramine produces a marked increase in fecal bile acid excretion and hepatic cholesterol synthesis without lowering the blood cholesterol concentration. This finding suggests that in these two species the ability to augment cholesterol synthesis may be adequate to replace the increased bile acid loss. The hypocholesterolemic effect of this resin has been reported in the cockerel, dog, rabbit, and in man; in these species the body's ability to augment cholesterol synthesis may be inadequate to replace the increased bile acid loss.

Carey and Williams (13) noted an 8-fold increase in fecal deoxycholic acid excretion in a normal human fed 10 g of cholestyramine daily. However, since they studied only one of the several bile acids excreted in man, an over-all estimation of the effect of the resin on cholesterol catabolism could not be made from their study. This resin has been used to relieve the pruritis associated with jaundice and incomplete biliary obstruction in man (13, 38, 39), and a lowering of blood bile acid concentrations with relief of pruritis has been demonstrated in such patients (13). Tennent, Siegel, Zanetti, Kuron, Ott, and Wolf (1) reported an increase in both the bile acid (2.6-fold) and the sterol (1.8-fold) output in a dog given very large doses of cholestyramine (25 g/day). Hashim and Van Itallie (9) found a 4- to 8-fold increase in fecal bile acid excretion with no change in neutral sterol output in one normal man and two hypercholesterolemic women, using nonisotopic methods. Since the intestinal bacteria may alter the structure of bile acids and sterols it is possible that incomplete recovery of the products of cholesterol catabolism may result with many of the analytical methods which have been used in these studies.

On a cholestyramine dose of 0.2 g/kg per day our patient had a 16% reduction in total serum cholesterol concentration within 7 days and a further decline to 80 % of pretreatment levels within 60 days. This compares favorably with the results of Bergen, Van Itallie, Tennent, and Sebrell (20 % fall), Horan, DiLuzio, and Etteldorf (12–44 % fall), and Hashim and Van Itallie (20–50 % fall). As noted by these authors, there was no escape from this effect during administration of the resin. The fecal excretion of bile acids in our patient increased 3.2-fold, which is comparable to that found in the dog (1) but less than that reported in man by Carey and Williams (8-fold) and by Hashim and Van Itallie (4- to 8-fold). It has already been mentioned that Carey and Williams studied only the excretion of deoxycholic acid and not the total bile acid and sterol excretion. The bile acid output in Hashim and Van Itallie's patients during the control period (150–289 mg/day) was lower than in our patient (287–353 mg/day), but the output during resin therapy (1243–1685 mg/day) was greater than in our patient (655–1305 mg/day). The discrepancy in these results may be due to differences in the methods employed or in variations in the responses of the subjects to the drug. The absence of any change in the unsaponifiable fraction in our patient during resin therapy confirms the report of Hashim and Van Itallie that the increase is in the bile acid excretion alone. This absence also demonstrated that there was no "loss" of bile acids into the sterol fraction during the extraction process we used.

During cholestyramine ingestion our patient excreted an additional 746 mg of bile acids per day which was adequate to account for the observed fall in serum cholesterol concentration during the first 7 days of this period. However, since the augmented fecal bile acid loss continued in spite of a leveling off in the blood cholesterol concentration, there must have been either an increase in

cholesterol synthesis or a shift of cholesterol from extravascular sites (or both) to compensate for the increased fecal loss. The appearance of the serum cholesterol specific activity–time decay curve (Fig. 2) supports this hypothesis, showing a more rapid rate of fall with the institution of cholestyramine and reaching, during the latter part of this period, an exponential rate of decline having a greater negative slope than before resin therapy. This phenomenon could be explained by a prompt increase in cholesterol catabolism (fecal bile acid excretion) followed by a compensatory increase in cholesterol synthesis and perhaps a shift of cholesterol from extravascular pools into the blood-liver pool, establishing a new dynamic steady state. However, since the blood cholesterol level declined, it is unlikely that any large shift of cholesterol into the blood-liver pool occurred during this period. Hashim and Van Itallie did note some softening and diminution in size of xanthomatous lesions in some of their patients after prolonged use of the resin, which would suggest a slow shift of cholesterol from these sites into other areas, probably the blood pool. Both the behavior of the serum cholesterol specific activity and the fecal bile acid excretion values demonstrate that in our patient there was a significant increase in the cholesterol turnover rate during the ingestion of cholestyramine.

Thus, it appears to be well established that cholestyramine acts primarily by binding bile acids in the intestinal lumen thereby enhancing their loss from the body and increasing the catabolism of cholesterol. In the doses commonly used in man there do not appear to be any significant side effects. Whether the increased catabolism of cholesterol and the resultant increased cholesterol turnover will be eventually beneficial in the reduction of atherosclerosis and its complications in man awaits long-term use of this drug and further investigation.

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